



Study of the *E. coli* elimination from urban wastewater over photocatalysts based on metallized TiO₂



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ABSTRACT

In this study, a series of photocatalysts based on TiO₂ was tested in the elimination of *Escherichia coli* (*E. coli*) from urban wastewater. Firstly, TiO₂ obtained by sol-gel method was modified by sulfation, and then gold or platinum nanoparticles were photodeposited on sulfated titania surface. Platinized samples were also prepared with different Pt content of 0.5 and 2 wt.%. The samples thus obtained were extensively characterized and it was found that sulfation considerably increases the S_{BET} value of TiO₂ and promotes the anatase phase formation; it was also found that 0.5 wt.% Pt-TiO₂ sample presents the lowest noble metal particle size and the best particle dispersion. All the photocatalysts synthesized have shown bactericidal effect and the results obtained by using bare and metallized TiO₂ were considerably better than the results obtained with the commercial TiO₂ P25 Evonic. Different light intensities were also evaluated in the photocatalytic tests and it was found that 120 W/m² leads to obtain the highest *E. coli* elimination from wastewater samples; however no total elimination of *E. coli* or other species of bacteria was achieved even after 5 h of photocatalytic treatment without catalyst. Total elimination of the *E. coli* was achieved after 3 h of photocatalytic reaction by using 120 W/m² of light intensity and 2 wt.% Pt-TiO₂ as photocatalyst; no bacterial regrowth was observed even after 72 h.

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1. Introduction

In many different regions around the world there are still many rivers containing an important pollution level coming from urban wastewater. This fact represents a serious problem for animal and human health; and for that reason, in the last decades, there has been an urgent need for the development of new and better treatments for drinking and wastewater. Different alternatives for water cleaning have been employed, conventional methods such as chlorination are very effective in the microbial elimination; however, chlorine can reacts with organic matter and other precursors to form regulated and emerging disinfection by-products, which can be associated with cancer or another human pathologies [1–3].

Photocatalysis is one of the most promising, safe, low cost and effective technologies for the polluted water treatment, it has been applied in the degradation of a wide range of environmental pollu-

tants [4–8] and currently it is recognized to be a good alternative for the elimination of different kind of bacteria [3,9–13–15].

A number of studies related with the *E. coli* inactivation by using photocatalysis have been addressed by different authors; most of them include the simulation of environmental conditions at the lab scale and by using commercial bacteria strain [9–12,14]. These studies have been very important to predict the behavior of the photocatalytic reactions; however, only a few researches have conducted studies by using real matrix or wastewater sources [3,15]. In order to determine the true effectiveness of the photocatalytic materials, it is very important to approach catalytic reactions by testing these materials in samples coming from real wastewater sources, which are polluted by different kind of bacteria and other organic contaminants.

The main objective of this work is to study the effectiveness of a series of photoactive materials in the treatment and inactivation of the enteropathogenic bacteria *E. coli*. This bacteria was used in these experiments due to its wide spread use as a faecal indicator and its resistance to the bactericidal effects of light irradiation relative to other bacteria [9]. Thus, the bactericidal effect of commercial TiO₂ P25 Evonic and other lab prepared photocatalysts obtained by TiO₂

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modification by sulfation and noble metal addition processes was evaluated.

The samples of urban wastewater used in the photocatalytic experiments were collected from the river “Jordán”, this is the main river located in the city of Tunja, Colombia. This river presents important contamination levels coming from the effluents of the industrial and human activities in this city. Currently, the treatment of this water source represents a priority; as the river course is very close to many urban residential neighborhoods.

2. Experimental

2.1. Photocatalysts preparation

Bare TiO₂ used as starting material was prepared by hydrolysis of titanium tetraisopropoxide (Aldrich, 97%) in iso-propanol solution (1.6 M) by the slow addition of distilled water (volume ratio isopropanol/water 1:1). The lab prepared TiO₂ powder was recovered by filtration and dried at 110 °C for 24 h. Afterwards, part of this material was submitted to calcination treatment at 650 °C for 2 h.

Sulfated TiO₂ (S-TiO₂) was obtained by immersion of the uncircled TiO₂ powder in a 1 M sulfuric acid solution under continuous stirring for 1 h, followed by filtration, drying, and calcination at 650 °C for 2 h.

Photodeposition of noble metals was performed over the S-TiO₂ material. Gold (III) chloride trihydrate (HAuCl₄·3H₂O, Aldrich 99.9%) or hexachloroplatinic acid (H₂PtCl₆, Aldrich 99.9%) were used as metal precursors for Au and Pt, respectively. Under an inert atmosphere (N₂), a suspension of S-TiO₂ in distilled water containing isopropanol (Merck 99.8%) which acts as sacrificial donor, was prepared. Then, the appropriate amount of metal precursor to obtain the desired nominal metal loading was added. Series of metallized photocatalysts were prepared with 0.5 weight total (wt.%) to TiO₂. Photochemical deposition of Pt or Au was then performed by illuminating the suspensions with an Osram Ultra-Vitalux lamp (300 W) with a sun-like radiation spectrum and a main emission line in the UVA range at 365 nm, using 120 min of photodeposition time. Light intensities on the TiO₂ surface were 0.15 and 60 W/m² for Au and Pt photochemical deposition, respectively. These intensities were selected based on previous studies where it was observed that under this irradiation it is possible to obtain optimized photocatalytic materials very effective in environmental remediation reactions [5,7,8]. The intensity of the incident UV-vis light on the solution was measured with a Delta OHM photodiometer HD2102.1. In order to study the effect of the metal loading over the *E. coli* photocatalytic treatment, a platinized sample with 2 wt.% of Pt was also prepared.

After noble metal deposition, the powders were recovered by filtration and dried at 110 °C overnight. The catalysts prepared are enlisted in Table 1.

Commercial TiO₂ P25 Evonic was used as received.

Table 1
Summary of the characterization results.

Photocatalyst	S _{BET} (m ² /g)	D _{Anatase} (nm)	Band gap (eV)	Binding energy (eV)	O/Ti	
					Ti 2p _{3/2}	O 1s
Bare-TiO ₂	11	17	3.30	458.5	529.8	1.96
S-TiO ₂	58	20	3.20	458.5	529.8	1.70
0.5 wt.% Au-TiO ₂	58	19	3.24	458.5	529.8	1.88
0.5 wt.% Pt-TiO ₂	49	20	3.20	458.4	529.6	1.91
2 wt.% Pt-TiO ₂	53	21	3.16	458.6	529.9	1.92
P25	51	22	3.23	458.5	529.8	1.87

2.2. Characterization of the photocatalysts

Specific surface area (S_{BET}) measurements were carried out by using low-temperature N₂ adsorption in a Micromeritics ASAP 2010 instrument. Degasification of the samples was performed at 150 °C.

Crystalline phase composition and degree of crystallinity of the samples were estimated by X-ray diffraction (XRD). XRD patterns were obtained on a Siemens D-501 diffractometer with Ni filter and graphite monochromator using Cu K α radiation. Anatase crystallite sizes were calculated from the line broadening of the main anatase X-ray diffraction peak (101) by using the Scherrer equation. Peaks were fitted by using a Voigt function.

Chemical composition and total noble metals content in the samples were determined by X-ray fluorescence spectrometry (XRF) in a Panalytical Axios sequential spectrophotometer equipped with a rhodium tube as the source of radiation. XRF measurements were performed onto pressed pellets (sample included in 10 wt.% of wax).

Gold and platinum particles sizes were evaluated by Transmission Electron Microscopy (TEM) in a Philips CM200 instrument. For this analysis the samples were dispersed in ethanol using an ultrasonicator and dropped on a carbon grid. Determination of the metal particle average diameter (\bar{d}) in the different samples was accomplished by counting particles in a high number of TEM images from different places of the samples. The following equation was used: $(\bar{d} \text{ nm}) = \sum di \times fi$. Where di is the diameter of the ni counted particles and fi is the particle size distribution estimated by: $fi = ni / \sum ni$. Where ni is the number of particles of diameter di .

Light absorption properties of the samples were studied by UV-vis spectrophotometry. The UV-vis DR spectra were recorded on a Varian spectrophotometer model Cary 100 equipped with an integrating sphere and using BaSO₄ as reference. Band-gaps values were calculated from the corresponding Kubelka-Munk functions, F(R ∞), which are proportional to the absorption of radiation by plotting $(F(R\infty) \times h\nu)^{1/2}$ against $h\nu$.

X-ray photoelectron spectroscopy (XPS) studies were carried out on a Leybold-Heraeus LHS-10 spectrometer, working with constant pass energy of 50 eV. The spectrometer main chamber, working at a pressure $< 2 \times 10^{-9}$ Torr, is equipped with an EA-200MCD hemispherical electron analyzer with a dual X-ray source working with Al K α ($h\nu = 1486.6$ eV) at 120 W and 30 mAC 1 s signal (284.6 eV) was used as internal energy reference in all the experiments. Samples were outgassed in the prechamber of the instrument at 150 °C up to a pressure $< 2 \times 10^{-8}$ Torr to remove chemisorbed water.

2.3. Wastewater sampling

Water samples were taken from a river highly polluted with urban wastewater, by following the standard methods for examination of water and wastewater [16].

2.4. Quantification of the microbial population

E. coli was selected as model microorganism; in order to determine the microbial population in the wastewater samples and the effectiveness of the photocatalytic treatment in the bacteria elimination; all the samples were analyzed before and after the photoreaction tests. The bacteria population was monitoring every hour during the photocatalytic reactions. For these assays the membrane filtration method Merck (method 9222) [16] was used. Coliform Chromocult® agar was employed as culture medium. The microbial concentration in the samples analyzed is reported in this work as CFU (Colony Forming Units)/100 mL of wastewater sample.

By using this method of analysis it was possible to determine total coliforms and other enterobacteriaceae in the samples.

2.5. Physicochemical analyzes

The Biochemical Oxygen Demand (BDO) was determined by following the Winkler method code TP0084.

Turbidity of the samples before and after treatment was also analyzed by using a Merck Turbiquant 1500 T turbidimeter and the results are reported as nephelometry turbidity units (NTU).

pH was measured by using a SI analytics Lab 850 pH-meter.

Alkalinity and chloride content of the wastewater samples before and after photocatalytic treatment were calculated by using the 2320 B-1995 APHA and 4500 Cl-B-1995 APHA methods, respectively [16]. All the physicochemical analyzes were carried out by double testing.

2.6. Bacteria photodegradation tests

All the experiments were carried out in a discontinuous batch of 400 mL pyrex reactor enveloped by an aluminum foil, containing 250 mL of wastewater sample. 1 g/L of photocatalyst weight was selected for this work taking into account that this value has demonstrated to be effective in the elimination of a wide range of organic pollutants as it was reported in some of our previous works. Cho et al. [11] have also found that 1 g/L is the best catalyst dosage for the *E. coli* elimination; on the contrary, Suri et al. [14] have reported the highest elimination of this bacteria by using 0.1 g/L of catalyst, as it was indicated by these authors the differences in the catalysts dosage, reported in many works can be explained by the reaction system used for the photocatalytic tests.

In this study the reaction system was illuminated through a UV-transparent Plexiglas® top window (threshold absorption at 250 nm) by an Osram Ultra-Vitalux lamp (300 W) with sun-like radiation spectrum and a main line in the UVA range at 365 nm. In order to favor the adsorption–desorption equilibrium, prior to irradiation the suspension was magnetically stirred for 10 min in dark. Magnetic stirring and constant oxygen flow of 35 L/h as an oxidant were used to produce a homogeneous suspension of the photocatalyst in the solution. A bubbler tank was used as a source of natural oxygen. The total reaction time was 5 h; after this time the treated samples were filtered and the microbiological and physicochemical analyzes were carried out. In order to study the bacterial regrowth after treatment, the samples were maintained outdoor at room temperature for 24, 48 and 72 h, after this time the samples were analyzed by membrane filtration method.

Taking into account that UV light has bactericidal effect, photolysis experiments, without catalysts were also carried out. Different light intensities were also evaluated, i.e. 30, 60 and 120 W/m².

In order to ensure the reproducibility of the photocatalytic treatments, selected photoreactions were carried out threefold, finding an error percentage of 0.1%.

It is important to comment that the microbial population concentration depends on environmental conditions such as pluviosity, present in the days close to the urban wastewater sampling; thus samples can be diluted in determined days. It can be one of the main challenges when the photocatalytic treatment is applied in real water source samples, and it is very important to consider these parameters in the reactor or pilot plant design. This research was developed between October and March, in these months the average CFU/100 mL of wastewater sample was 2.9×10^5 , 7.8×10^5 and 3.7×10^5 for *E. coli*, total coliforms and other enterobacteriaceae, respectively. The initial bacteria population concentration is an important factor to consider when the photocatalytic processes will be applied at large scale.

Finally, the photocatalyst showing the best performance in the *E. coli* elimination was selected to conduct recycling tests. The catalysts were recovered after photoreaction by filtration, washing with distilled water and dried at 170 °C for 2 h.

3. Results and discussion

3.1. Characterization results

Some characterization results for the photocatalysts studied in this work have been previously reported [5,7,8]; however, in order to correlate these results with the photocatalytic behavior of the materials tested, the most relevant data have been included in this manuscript. Table 1 summarizes the main characterization results.

As it can be observed in this table, lab prepared TiO₂ (bare-TiO₂) presents the lowest specific surface area (S_{BET}), this is due to particle sintering during the calcination stage at the relatively high temperature used in the photocatalyst synthesis. It can also be observed that the sulfation of TiO₂ before calcination protects the titania surface, inhibiting particle aggregation; thus, after sulfation TiO₂ sample keeps its surface area in a higher degree. With the metal photodeposition the surface area slightly decreases due to pore blocking by metal nanoparticles on the titania surface. This effect is less marked in the Au-TiO₂ sample and in the platinized catalyst prepared with 2 wt.% of Pt; as in these samples the metal particles are higher than the platinum particles present in the catalysts prepared with the lowest metal content, as it can be seen in Fig. 1.

Fig. 1 shows selected TEM images of Pt-TiO₂ samples prepared with (A) 0.5 and (B) 2 wt.% of Pt content and (C) Au-TiO₂. In these images gold and platinum particles can be seen as dark spherical spots placed on the larger anatase particles. In the 0.5 wt.% Pt-TiO₂ photocatalyst, the platinum particles are more homogeneously distributed all over the titania surface, being the average Pt particle size 4–5 nm. For the catalyst prepared with 2 wt.% of platinum loading most Pt deposits are present as large agglomerates with sizes larger than 10 nm (Fig. 1(B)). This high degree of agglomeration prevented any attempt of particle size distribution determination. In the case of the Au-TiO₂ sample, gold particles present sizes larger than 10 nm and the metal particles are very heterogeneously distributed on surface.

Cristallinity of the samples was determined by XRD and the patterns obtained are presented in Fig. 2. As it can be seen, bare TiO₂ sample presents anatase and rutile phases, identified by the main XRD peaks at 25.25° and 27.44°, respectively, in a ratio about 90% anatase and 10% rutile. In the case of the sulfated and metallized samples, only anatase phase was identified. As it has been reported, this is mainly due to the protective effect of the sulfation pre-treatment, which inhibited the formation of rutile phase of TiO₂ during the calcination [7,17–19]. In metallized samples, no diffraction peaks for Au or Pt were detected.

XRF determined the real metal content in the analyzed materials: 0.32, 0.30 and 1.25 wt.% for Au-TiO₂, 0.5 wt.% Pt-TiO₂ and 2 wt.% Pt-TiO₂ samples, respectively; thus indicating an incomplete reduction of the metal precursors on TiO₂ surface during the photocatalysts synthesis.

The UV-vis DR spectra of the catalysts analyzed are shown in Fig. 3. Typical absorption band edge of the TiO₂ at around of 400 nm can be seen in all spectra, and no significant changes in the UV zone were observed after metallization. However; the metallized samples present a higher absorption in the visible zone (400–800 nm); as expected due to the color of these samples, purple or gray for Au-TiO₂ and Pt-TiO₂, respectively. The highest visible absorption was observed in the 2 wt.% Pt-TiO₂ sample. The band gap energies for the samples analyzed are between 3.16 and 3.30 eV (Table 1); this

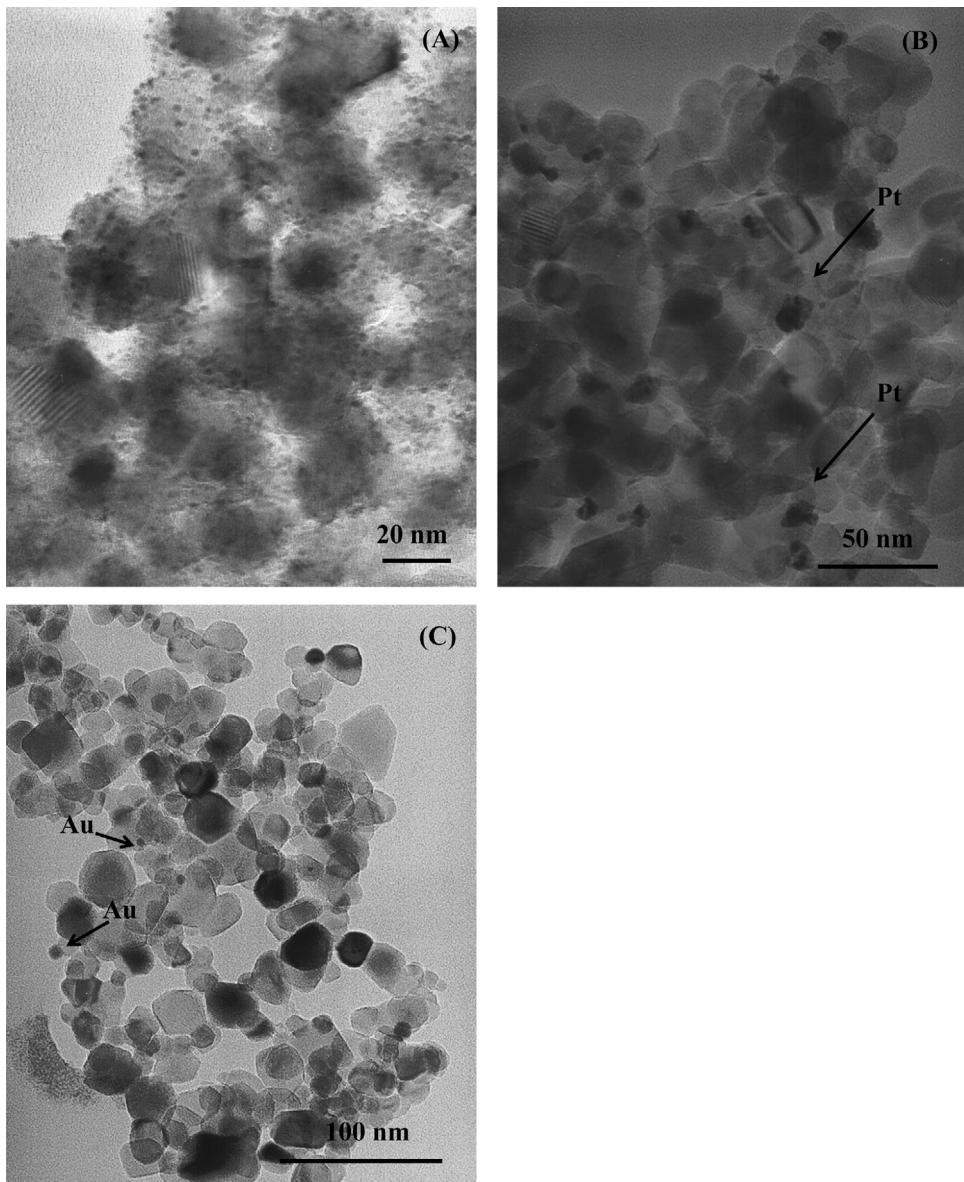


Fig. 1. TEM images of metallized catalysts. (A) 0.5 wt.%; (B) 2 wt.% Pt-TiO₂ and (C) Au-TiO₂.

value slightly decreases after sulfation and metallization processes. The lowest band gap value was observed in the 2 wt.% Pt-TiO₂ photocatalyst.

XPS measurements were also performed and a summary of these results is reported in Table 1. The Ti 2p core peaks exhibit a main component at around 458.4 ± 0.1 eV (Ti 2p_{3/2}) in all the samples; representative of the Ti⁴⁺ ions in TiO₂ lattice. On the other hand, in the O 1 s region, a peak located at 529.6 ± 0.2 eV can be observed for all the samples, assigned to oxygen atoms in the TiO₂ lattice. This peak is asymmetric with a shoulder located at higher binding energies; assigned to surface OH⁻ groups.

By XPS it was possible to identify the presence of reduced and oxidized species of Au and Pt (spectra are not shown for the sake of brevity). It can be noted that the photodeposition process is not able to reduce the totality of the metal precursor, as it was also possible to determine from XRF analyzes.

By XPS it was also possible to estimate the superficial O/Ti ratio (Table 1). S-TiO₂ sample presents the lowest value (i.e 1.70), due to the creation of surface oxygen vacancies coming from the dehy-

droxylation process, which takes place during the calcination at high temperature [7,17].

3.2. Bacteria elimination tests

For these initial tests 30 W/m^2 of light intensity was selected, as this intensity is very close to the annual average solar light intensity in Tunja, the Colombian town where this study was carried out. So, improving photocatalytic treatments under these conditions, will allow us in the future to use solar light radiation in the photocatalytic reactions focused on environmental remediation.

The reduction of the turbidity in the samples was the first evident physical change observed in the wastewater after photocatalytic treatment, thus, the turbidity values were reduced to about half after treatment for all the tested photocatalysts. In Fig. 4, it is possible to identify qualitatively the changes in the turbidity before and after photocatalytic treatment over commercial TiO₂ P25 Evonic, where the turbidity values correspond to 28.90 and 13.00 NTU respectively.

Table 2

Physicochemical analyzes results before and after photolysis or photocatalytic treatment.

Photocatalyst	Light intensity (W/m ²)	Before and after treatment	pH	Alkalinity (mg CaCO ₃ /L)	Cl ⁻ (mg/L)	BDO (mg/L)
P25	30 W/m ²	BT	7,55	279	140,22	0,57
		AT	8,04	180,18	140,22	0,14
Bare-TiO ₂		BT	6,87	84,08	59,64	5,00
		AT	8,45	63,06	59,65	0,72
S-TiO ₂		BT	6,53	70,07	39,76	1,43
		AT	7,57	28,02	39,76	0,72
0,5 wt.% Au-TiO ₂		BT	6,98	63,06	39,76	4,64
		AT	7,95	45,54	39,76	0,40
0,5 wt.% Pt-TiO ₂		BT	6,98	63,06	39,76	3,58
		AT	7,94	42,04	39,76	0,30
0,5 wt.% Pt-TiO ₂	120 W/m ²	BT	7,24	273,78	112,02	1,17
		AT	8,35	237,27	112,02	0,75
2 wt.% Pt-TiO ₂		BT	7,20	273,78	140,02	1,65
		AT	8,30	182,52	140,02	0,43
Photolysis at Different Light Intensity						
30 W/m ²		BT	7,80	291,85	140,02	2,78
		AT	8,1	270	140,02	0,28
60 W/m ²		BT	7,24	49,04	19,88	3,57
		AT	8,72	45,54	19,88	0,64
120 W/m ²		BT	7,24	365,04	120,02	3,57
		AT	8,43	237,27	120,02	0,50

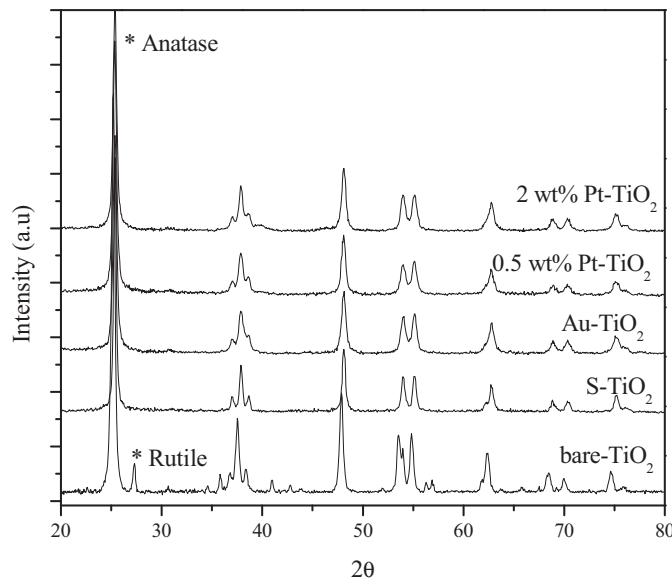


Fig. 2. XRD patterns for the photocatalysts analyzed.

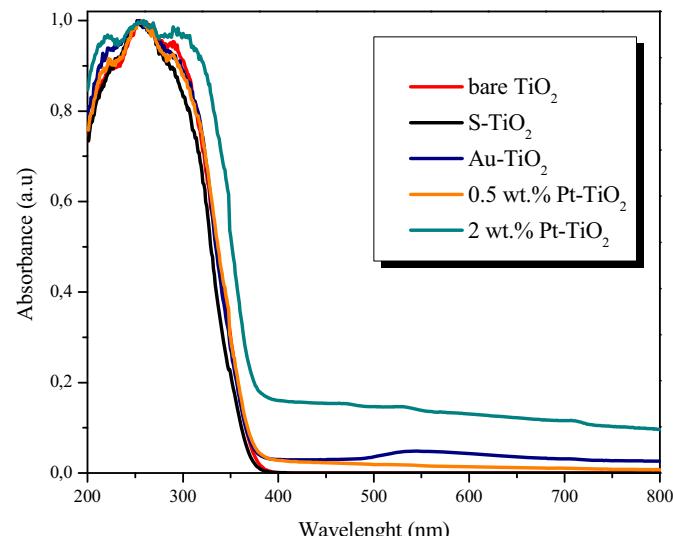


Fig. 3. UV-vis DR spectra of photocatalysts analyzed.

It was also found that alkalinity values in the wastewater samples are mainly represented by CaCO₃ and HCO₃ species, this value decreases after photocatalytic treatment, as it can be seen in Table 2, it can be due to the adsorption of these ions on the photocatalysts surface during treatment. It can be also observed in Table 2 that pH increases after photolysis or photocatalytic reactions; it can be explained due to the formation of by-products coming from protein oxidation during the bacteria inactivation.

The BDO before and after treatment was also measured; these results are presented in Table 2. As it can be observed, this parameter considerably decreases as a result of the photocatalytic treatment; thus indicating a bactericidal effect of the process.

The effectiveness of the catalysts prepared was evaluated in the elimination of *E. coli* and other microorganisms present in urban wastewater; the CFU/100 mL observed after the photocatalytic treatment carried out under 30 W/m² of light intensity are shown in Fig. 5.



Fig. 4. Selected images of the turbidity changes in wastewater sample before (left) and after (right) of the photocatalytic treatment over P25.

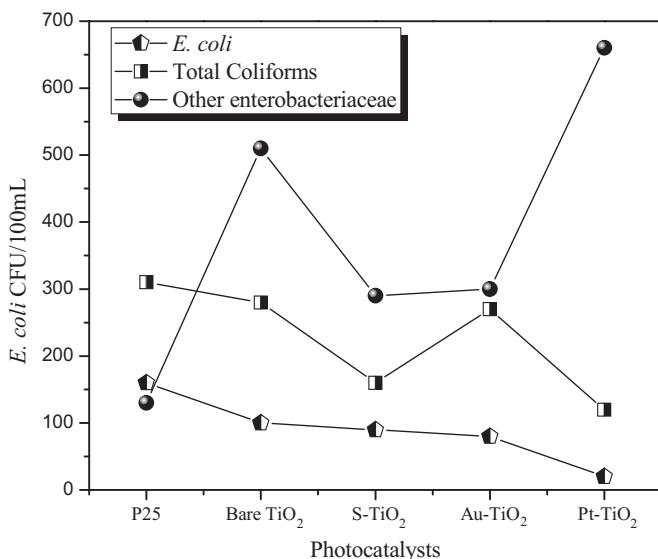


Fig. 5. CFU/100 mL of microorganisms after wastewater treatment over photocatalysts analyzed, using 30 W/m² of light intensity and 5 h of total reaction time.

As it can be seen in this figure, *E. coli* has the highest susceptibility to be eliminated by photocatalytic process. Other bacteria are more resistant to this treatment, probably due to the competition of different bacteria species for the OH[•] radicals in solution.

In the case of *E. coli* and total coliforms, the lowest effectiveness in the phototreatment was observed over the commercial TiO₂ P25 Evonic. Lab prepared TiO₂ (bare TiO₂) is more effective in the elimination of these species of bacteria than P25. The bactericidal effect of the bare TiO₂ increases after sulfation, that can be due to the presence of only anatase phase in the S-TiO₂, which has been considered the most active phase of titania in photocatalytic processes, as well as to a higher surface area value for this sample [20,21]. The noble metal addition (gold or platinum) also improves the effectiveness of the sulfated TiO₂, thus, the lowest CFU value for *E. coli* and total coliforms were obtained by using the 0.5 wt.% Pt-TiO₂ as photocatalyst.

The improvement of the TiO₂ photoefficiency after sulfation and noble metal addition may be due to: (i) The sulfation process has a protective effect on titania surface during the calcination at high temperature, thus, after sulfation the specific surface area of TiO₂

increases and sulfation leads to obtain 99% anatase phase and to create oxygen vacancies on titania surface, as it can be observed in Table 1. It has been reported that oxygen vacancies and noble metal nanoparticles on titania surface act as sink for electron photogenerated during the photocatalytic reaction, thus reducing the e⁻ h⁺ recombination rate and therefore leading to a more effective photocatalytic treatment [22–24] for bacteria in the specific case of this work; (ii) gold and platinum nanoparticles can act as potent antibiotics by itself, as it has been reported by Zhao et al. [25], these authors have explained the mechanism of antibiotic action mainly due to the dissipation of membrane potential and the elevation of adenosine triphosphate (ATP) levels, these authors also have found that Pt contents between 10 and 65% leads to increase the bactericidal effect of this metal in the *E. coli* elimination.

3.2.1. Effect of the light intensity

It is known that UV light has bactericidal effect by itself and it has been explained that the damage caused by UV light is mainly due to its absorption by cellular components called intracellular chromophores, the best known intracellular chromophore is L-tryptophan. In the presence of oxygen, UV irradiation contributes to the generation of reactive oxygen species (ROS), which induce oxidative stresses that damage the cell membranes and components [3,12]. In order to study the effect of the light intensity over the elimination of the bacteria in urban wastewater samples and also in order to select the best light intensity in the photocatalytic treatment for *E. coli*, in this work different intensities in photolysis reaction without catalyst were tested (i.e 30, 60 and 120 W/m²). The results obtained are presented in Fig. 6. As expected, the temperature inside of the reaction system increases with the light intensity; this is due to the closeness of the UV lamp to the batch reactor. The temperatures inside of the suspension of wastewater were 38, 47 and 54 °C for 30, 60 and 120 W/m² of light intensity, respectively.

The results obtained in this stage of the work have indicated that, as expected, the *E. coli* inactivation level increases with the light intensity; thus, the highest microorganisms elimination was obtained by using 120 W/m²; using this intensity the lowest CFU/100 mL of *E. coli* and other bacteria were detected, after 5 h of photocatalytic reaction. The highest effectiveness obtained in the *E. coli* elimination at this light intensity is related with a combined effect between UV light and high temperature, which contributes to increase the bacteria elimination. This result implies that the

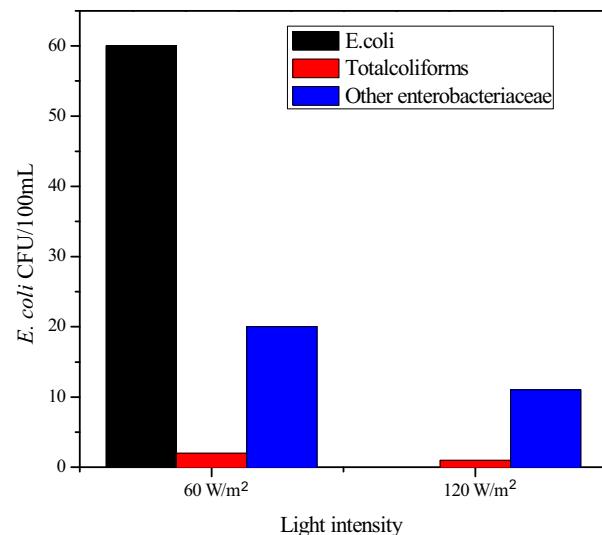
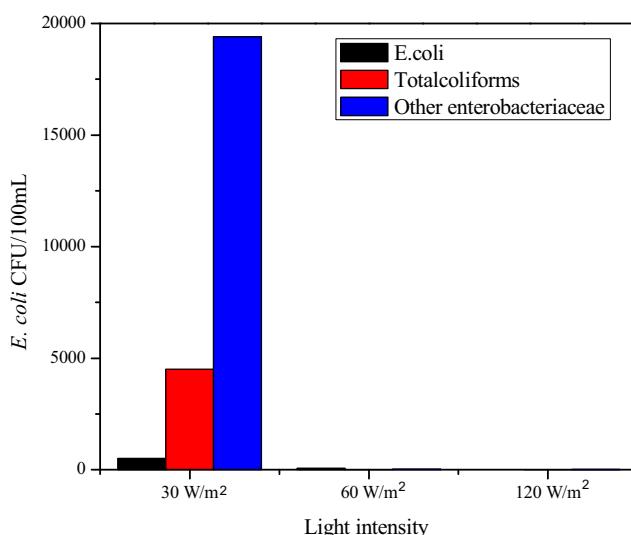


Fig. 6. CFU/100 mL of microorganisms after photolysis by using different light intensity and 5 h of total reaction time.

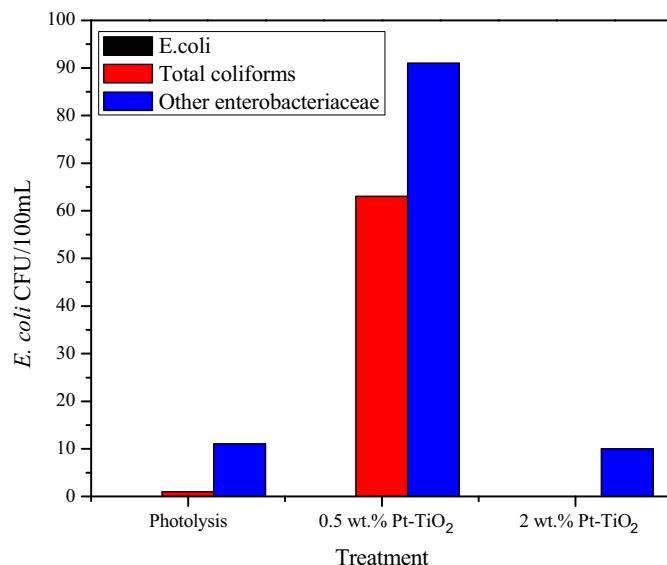


Fig. 7. CFU/100 mL of microorganisms after photocatalytic treatment over Pt-TiO₂ catalysts with different platinum content at 120 W/m² light intensity and 5 h of total reaction time.

temperature increasing changes the microorganism susceptibility, as it has been also reported by Cho et al. [11].

3.2.2. Effect of the Pt content

Taking into account that 120 W/m² light intensity leads to obtain the highest elimination of *E. coli* and total coliforms present in the wastewater samples, and in order to study the effect of the noble metal content over the bactericidal effect of TiO₂, series of photocatalytic tests using catalysts prepared with 0.5 and 2 wt.% of Pt content was also tested in the wastewater treatment at 120 W/m². These results are shown in Table 2 and in Fig. 7. It general, it was found that the increase in the Pt loading significantly increases the bactericidal effect of TiO₂; similar results were observed by Suri et al. [14], these authors have studied the *E. coli* inactivation over platinized TiO₂ by using solar light, a metal content between 0.5 and 5 wt.% were evaluated, and it was found that the bactericidal effect increases with the metal content, thus, the highest efficiency in the *E. coli* elimination (no total elimination), was obtained for these authors over the photocatalyst prepared with 5 wt% of Pt.

In the present study, and as it can be seen in Fig. 7, the efficiency in the bacteria elimination obtained over the 2 wt.% Pt-TiO₂ catalyst was higher than the achieved over the 0.5 wt% Pt-TiO₂ catalyst. It can be explained taking into account that in the 0.5 wt.% Pt-TiO₂ sample, it was observed the highest number of Pt nanoparticles (Fig. 1(A)), it has been reported that in some cases these nanoparticles homogeneously distributed can obstruct the effective adsorption of the substrate to be degraded on TiO₂ surface, thus reducing the effectiveness of the photocatalytic treatment [7]. In the case of the bacteria analyzed in the present work, the reduction in the effectiveness of the photocatalytic treatment using 0.5 wt.% Pt-TiO₂ as catalyst can be also related with the size and shape of these microorganisms, this is because bacteria are more than 30 times bigger than TiO₂ particles [26], so the presence of a high number of Pt nanoparticles on TiO₂ surface, can also obstruct the diffusion of bacteria in solution. In the case of 2 wt.% Pt-TiO₂, the highest agglomeration of Pt particles is observed and in this sample there are sections on titania surface partially empty, where an increasing bacterial adsorption on the surface of photocatalyst can occurs.

This last conclusion could be contradictory if one observes the results obtained by using the Au-TiO₂ photocatalyst (Fig. 5), which

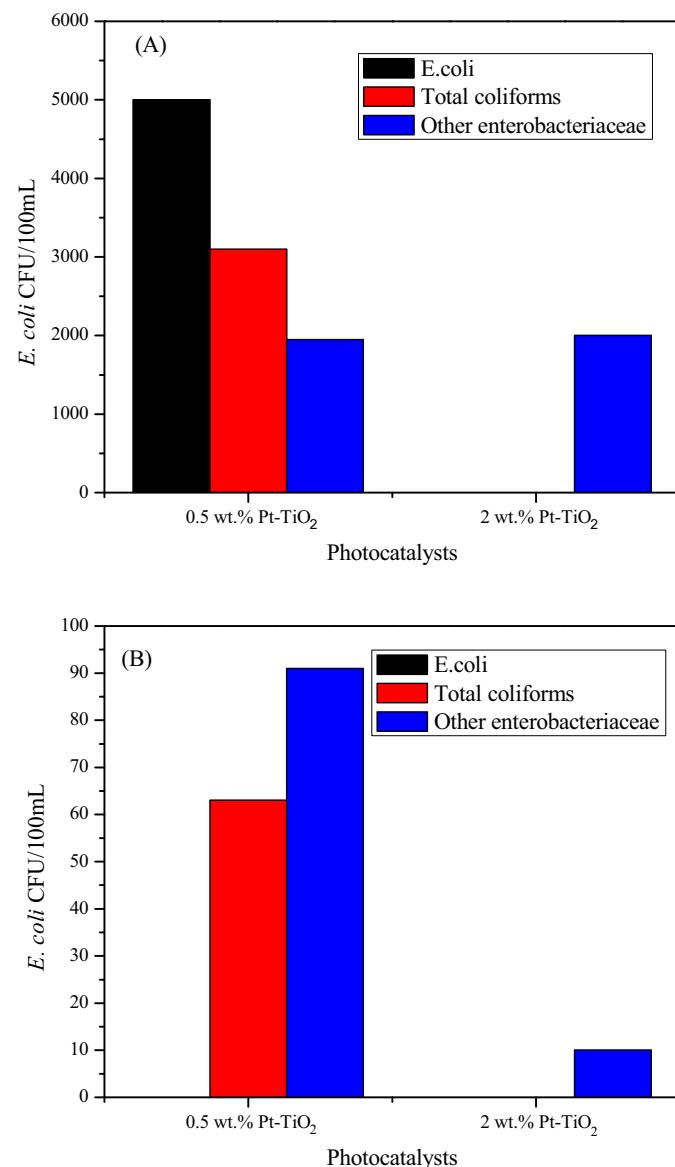


Fig. 8. CFU/100 mL of microorganisms after photocatalytic treatment over Pt-TiO₂ catalysts with different platinum content at 120 W/m² light intensity and 3 h (A) and 5 h (B) of total reaction time.

also present particles with high sizes heterogeneously distributed on titania (Fig. 1(C)), however, in this sample the photodeposition of metal during the synthesis processes was less effective leading to obtain a low number of gold particles deposited on surface compared with the platinized sample prepared with 2 wt.% of Pt. In addition, it is important to note that the 2 wt.% Pt-TiO₂ photocatalyst presents the highest absorption in the visible region and the lowest band gap value, as it was observed by UV-vis DRS analyzes (Fig. 3), these characteristics can favor the photoactivity of TiO₂ thus increasing their effectiveness in the photocatalytic treatment.

It is also important to indicate that by using 2 wt.% Pt-TiO₂ as photocatalyst the total reaction time necessary to the total elimination of *E. coli* was significantly reduced, this objective was achieved in just 3 h. A comparative graph of the results obtained after 3 and 5 h of photoreaction is presented in Fig. 8 (A) and (B), respectively. It is also important to note that in this test for the first time in the present research, the entire elimination of total coliforms present in the samples treated was achieved. So, it appears that a combined effect between the highest UV light intensity and 2 wt.% of Pt

content leads to increase the sensitivity of other microorganisms present in the wastewater samples analyzed, to be eliminated by heterogeneous photocatalysis.

No bacterial regrowth of *E. coli* or total coliforms was observed even after 24, 48 or 72 h, thus showing the effectiveness of the antibacterial treatment applied, by using 120 W/m² of light intensity and 2 wt.% Pt-TiO₂ as photocatalyst.

Furthermore, as it was indicated in experimental section, recycling tests by using the 2 wt.% Pt-TiO₂ photocatalyst and 120 W/m² of light intensity were carried out. After recovering and sterilization this material shows good stability, no deactivation was detected; thus, the photocatalytic activity of this catalyst was maintained even after three cycles of photoreaction, leading in all the cycles to achieve the entire elimination of *E. coli* and total coliforms after 3 h of photoreaction time, any regrowth of these microorganisms after the treatment applied to the urban wastewater samples, was observed, even after 72 h.

On the other hand, in order to discriminate the contribution of heating, photolysis and photocatalysis to disinfection, photolysis and photocatalytic tests were carried out under thermostatic conditions. These experiments were conducted at 18° C. As expected, when the experiments were carried out under controlled temperature, the effectiveness of the treatments in the bacteria elimination was lower than the obtained without temperature control, this effectiveness follows the next order: 30 W/m² < 120 W/m² < 2 wt.% Pt-TiO₂ + 120 W/m². From these results it was possible to corroborate the bactericidal effect of the UV light itself, but it was also evidenced that as expected, the presence of a photocatalyst in the reaction system is necessary for bacteria elimination improving.

In general, in this work it was found that the modification of TiO₂ by sulfation and noble metal addition have a beneficial effect over their photocatalytic activity, but it is important to note that under the experimental conditions used in this work the effectiveness of the photocatalysts analyzed is strongly influenced by the initial concentration of microorganisms present in the sample to be treated; in this sense it would be interesting to know the bacteria adsorption capacity on the photocatalysts surface in dark during different periods of time and to analyze samples coming from commercial strain, these studies are currently under development and it will be presented in future work.

4. Conclusions

E. coli and other total coliforms present in urban wastewater samples are sensitive to be eliminated by photocatalysis treatment.

Sulfation and noble metal addition lead to increase the bactericidal effect of lab prepared TiO₂, thus obtaining higher inactivation for *E. coli* than the obtained on commercial titania P25 Evonic.

Light intensity is an important factor influencing the *E. coli* elimination, the highest elimination of this microorganism was achieved by using 120 W/m² of light intensity, 5 h of reaction time and no catalyst. This is mainly due to a positive combined effect between UV light and high temperature. However, other bacteria present in the wastewater samples can survive after UV treatment at 120 W/m².

Finally, it was possible to determine that the best reaction conditions for an effective photocatalytic treatment, focused on the total elimination of *E. coli* and total coliforms present in urban wastewater samples were 3 h of reaction time, 120 W/m² of light intensity and the use of 2 wt.% Pt-TiO₂ as photocatalyst.

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References

- [1] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, *Mutat. Res. Rev. Mutat. Res.* 636 (2007) 178–242.
- [2] R.J. Buil, D.A. Reckhow, X. Li, A.R. Humpage, C. Joll, S.E. Hrudey, *Toxicology* 286 (2011) 1–19.
- [3] A. Costa, M. Lepretti, L. Rizzo, I. Caputo, V. Vaiano, O. Sacco, W. Silva, D. Sannino, *Sci. Total Environ.* 554–555 (2016) 1–6.
- [4] E. Kordouli, K. Bourikas, A. Lycurghiotis, C. Kordulis, *Catal. Today* 252 (2015) 128–135.
- [5] J.J. Murcia, M.C. Hidalgo, J.A. Navío, V. Vaiano, D. Sannino, P. Ciambelli, *Catal. Today* 209 (2013) 164–169.
- [6] V. Vaiano, O. Sacco, G. Iervolino, D. Sannino, P. Ciambelli, R. Liguori, E. Bezzeccheri, A. Rubino, *Appl. Catal. B* 176–177 (2015) 594–600.
- [7] J.J. Murcia, M.C. Hidalgo, J.A. Navío, J. Araña, J.M. Doña-Rodríguez, *Appl. Catal. B* 150–151 (2014) 107–115.
- [8] V. Vaiano, G. Iervolino, D. Sannino, J.J. Murcia, M.C. Hidalgo, P. Ciambelli, J.A. Navío, *Appl. Catal. B* 188 (2016) 134–146.
- [9] S. Helali, M. Polo-López, P. Fernández-Ibáñez, B. Ohtani, F. Amano, S. Malato, C. Guillard, *J. Photochem. Photobiol. A* 276 (2013) 31–40.
- [10] A.G. Rincón, C. Pulgarin, *Sol. Energy* 77 (2004) 635–648.
- [11] M. Cho, H. Chung, W. Choi, J. Yoon, *Water Res.* 38 (2004) 1069–1077.
- [12] M. Kacem, G. Plantard, N. Wery, V. Goetz, Chin. J. Catal. 35 (2014) 1571–1577.
- [13] I. García-Fernández, I. Fernández-Calderero, M. Polo-López, P. Fernández-Ibáñez, *Catal. Today* 240 (2015) 30–38.
- [14] R.P.S. Suri, H.M. Thornton, M. Muruganandham, *Environ. Technol.* 33 (2012) 1651–1659.
- [15] J.A. Herrera Melián, J.M. Doña Rodriguez, A. Viera Suarez, E. Tello Rendón, C. Valdes do Campo, J. Araña, J. Pérez Peña, *Chemosphere* 41 (2000) 323–327.
- [16] American Public Health Association (APHA), *Standard methods for examination of water and wastewater*, 22th edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, D.C., United States, 2012.
- [17] G. Colón, M.C. Hidalgo, J.A. Navío, *Appl. Catal. B* 45 (2003) 39–50.
- [18] D. Li, H. Haneda, S. Hishita, N. Ohashi, N.K. Labhsetwar, *J. Fluorine Chem.* 126 (2005) 69–77.
- [19] K. Okazaki, Y. Morikawa, S. Tanaka, K. Tanaka, M. Kohyama, *J. Mater. Sci.* 40 (2005) 3075–3080.
- [20] A.L. Linsebigler, G.Q. Lu, J.J.T. Yates, *Chem. Rev.* 95 (1995) 735–758.
- [21] M.C. Hidalgo, G. Colón, J.A. Navío, *J. Photochem. Photobiol. A* 148 (2002) 341–348.
- [22] M.I. Litter, *Appl. Catal. B* 23 (1999) 89–114.
- [23] T.Y. Tan, C.K. Yip, D. Beydoun, R. Amal, *Chem. Eng. J.* 95 (1–3) (2003) 179–186.
- [24] H.M. Coleman, K. Chiang, R. Amal, *Chem. Eng. J.* 113 (2005) 65–72.
- [25] Y. Zhao, C. Ye, W. Liu, R. Chen, X. Jiang, *Angew. Chem. Int. Ed.* 53 (2014) 8127–8131.
- [26] A.K. Benabbou, Z. Derriche, C. Felix, P. Lejeune, C. Guillard, *Appl. Catal. B* 76 (2007) 257–263.